

NON-ULCERATING ORIENTAL SORE :  
THE CULTURAL CHARACTERISTICS OF  
THE PARASITE AS COMPARED WITH  
A NEW SIMILAR PARASITE IN  
*ERTHESINA FULLO* (THUMB), A  
PENTATOMID BUG

BY

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In tropical and sub-tropical countries several peculiar types of skin lesion occur endemically and often limited in distribution to certain districts. These skin lesions have a very varied nomenclature but are loosely grouped under the name Oriental sore. In November, 1909, a brief differentiation between three types of Oriental sore was first made, and for the purposes of this communication, which is confined to experimental work on the parasites of the non-ulcerating type, as compared with a new and a similar parasite in *Erthesina fullo*, the three types of Oriental sore will be termed (1) the non-ulcerating Oriental sore, (2) the superficial flat Oriental ulcer, (3) the deep-seated Oriental boil. It is of interest to note that Thomson and Balfour\* confirm the presence of the first type in Egypt, and wholly agree with me as to the possibility of there being different varieties of *Leishmania* hitherto undifferentiated.

Recent experience has shown that the non-ulcerating type of Oriental sore is in India and neighbouring countries a not uncommon affection. In a mixed population of over 2,000 patients from all parts of Northern, Western, Eastern and Central India, Burma and Assam, who sought anti-rabic treatment at the Pasteur Institute of India, seven cases of non-ulcerating Oriental sore were seen in

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sixteen months. Neither of the two other types occurred, though all three were sought for. From the characteristic painless indolent nature of this cutaneous lesion, it can, however, readily be understood how easily it is overlooked unless seen in an advanced condition, or in a site such as the face or hand under frequent observation. It is thus probable other early cases in this number of patients escaped notice.

The history of these seven cases is as follows:—

CASE I.—Boy, — Singh, aged 19, native from Patiala State. Patient presented a large swelling on the right cheek, simulating a gumboil. In the centre of this swelling was a slightly raised area, measuring  $\frac{1}{2}$  inch by  $\frac{3}{8}$  inch, pale straw in colour, and covered with fine papery scales. The edge of this raised area was slightly indurated and could be differentiated by the touch from the soft resilient raised patch.

The upper right eyelid presented a small circular raised nodule, reddish in colour, about  $\frac{1}{4}$  inch in diameter. The left forearm presented four small raised nodules, one exactly like that on the eyelid, the other three resembling the area on the face in all details. There was the scar of an old ulcer on the back of the left forearm, said by patient to have resulted from an injury. No other person in his family shows or has ever shown similar cutaneous lesions.

History of the lesions.—The spot on the cheek first appeared fourteen months previously. It started as a minute itching red spot, which in a few weeks became anaesthetic to the touch and gradually extended to its present size. Six months later patient noted a similar spot appearing on the front of his right forearm. The other lesions on the forearm and eye developed subsequently.

Patient is in the habit of working in the open air, inspecting fields, etc., at his village. He often sleeps in the open during the day. He was shown specimens of ticks and biting flies to see if he could recognise them. He recognised Tabanids, Hippoboscidae and Haematopota as common biting flies of his district attacking animals. Patient does not remember being bitten by either ticks or flies, but states he has seen Tabanids bite persons as well as animals. The bite has been described to him as not being painful, but bleeding freely after a few seconds' smarting.

He described other flies in his district that bite, especially very small species in the neighbourhood of hill streams. These probably are Simulium and mosquitos.

Smears were taken from each spot, and also of peripheral blood from the right arm and ear. The blood clotted with unusual rapidity.

In all slides from the spots the mononuclear cells were found distended with Leishmania-like parasites. There were many free forms, those in the mononuclear cells were mainly oval and vacuolated. The small torpedo-form described in Delhi boil was comparatively rare.

In the films of peripheral blood, malarial parasites alone were seen.

Repeated punctures healed up invariably within a few days. The patient's spleen and liver were slightly enlarged.

CASE II.—Miss C., European, Medical Missionary from Gujrat, aged 24. Presented a small raised area of pale straw colour on the forehead. This area was smooth and hairless over the greater part of its surface, the periphery was covered with fine paper-like scales of epithelium. The margin was sharply defined by a fine reddish indurated line of inflammation, which faded for about  $\frac{1}{4}$  inch into the normal surrounding skin. The affected area measured  $\frac{1}{2}$  inch by  $\frac{3}{8}$  inch. The lesion was completely anaesthetic. The red margin was sensitive. Miss C. names the affection 'Monghyr Phora,' this being the name given to this lesion in her district.

The history of the case is as follows :—Miss C. first noted a small, itching, red pimple, like the bite of an insect, on her forehead thirteen months previously. This shortly became anaesthetic, but gradually increased. When the area was  $\frac{1}{2}$  inch in diameter it was painted with pure carbolic. This treatment did not arrest the progress of the lesion. A second minute pimple of the same type as the first appeared on the point of the elbow some months later. Patient states she has had no fever for eight years. She presents no enlargement of liver or spleen. She has, however, noticed that since the appearance of the spot on the forehead she has been feeling ill.

Examination of blood films from punctures of each spot showed the mononuclears distended with parasites as in the previous case. This blood clotted very rapidly. The peripheral blood showed no parasites of any kind.

Miss C., when asked if she recognised three or more types of Oriental sore, stated three were recognised in her district : (1) 'Monghyr Phora,' above described, a non-ulcerating form; (2) a large superficial ulcer, common on the hands, wrists, ankles, and feet, whose floor was composed of exuberant granulations covered with pus and débris—this type is known locally as 'Chambal'; (3) a deep crater with raised sides, the former filled with and the latter undermined by foul pus and epithelial débris, this is known as 'Delhi boil.'

Popular belief is that the cause of the first type is the bite of the sand-fly or mosquito.

CASE III.—Mr. E., European, aged 45, from Scinde. Patient presents multiple infection with non-ulcerating Oriental sore. The first appeared at Gumbat, outside Kohat, as a minute itching pimple on the forehead. This has slowly increased for fourteen months, is of pale straw colour, and raised above the surface of the adjacent skin, from which it is separated by a red thickened edge. The affected area is under  $\frac{1}{2}$  an inch in its greatest diameter.

A second spot appeared on the back of the forearm eight months ago, this is similar to the first, but encroaches on to an old healed scar, which seems to have limited its spread on that side. There are seventeen other scars on this forearm, patient states they are different from ulcerating 'Scinde sore.'

A third similar area was noted on the front of the left wrist. It was larger but similar to the previous two described, covered with fine papery scales, and completely anaesthetic. Patient first had a small ulcerating 'Scinde sore,'  $2\frac{1}{4}$  years previously on the forehead, this healed with difficulty and left a small, smooth, white, depressed scar. Four similar 'Scinde sores' developed later on the forehead, and fourteen sores on and around the right elbow joint. The scars were most distinct, many of them had a brown tinge round their edges. The raised lesions were examined for parasites and a condition found as in the previous cases. The blood clotted rapidly. There was but little enlargement of the spleen. Patient had a malarial history, but no parasites were found in the peripheral blood. He describes three forms of sore in Scinde : (1) the flat indolent and often multiple ulcer, known as 'Scinde sore,' which is the commonest met with; (2) the deep-seated affection like a deep boil or carbuncle; (3) the non-ulcerating form, comparatively rare. Patient asked, as he had now had both the first and third varieties, if he was liable to contract the second.

Patient states the sore on the forehead appeared at the beginning of the hot weather. He attributes these to the bite of either mosquitos or sand-flies, which are commoner than other pests outside Kohat. None of his servants or friends were suffering from the same disease.

CASE IV.—Ephraim, son of W., native Christian from Gujrat, aged 7 years 2 months. Patient presents a small raised pale brown area the size of a three-penny bit,  $\frac{1}{4}$  inch from the lower edge of the right nostril. The skin in the centre is smooth, and is seen under a lens to be devoid of fine hairs. The edges are covered with fine papery scales, the margin of the affected area is dark and slightly more indurated than the adjoining healthy tissue.

This spot first appeared seventeen months previously at the commencement of the hot weather, as a small itching point in the skin which gradually increased in size. Though anaesthetic at the time of examination, patient's parents state the child complains of intense itching in the affected area at intervals of many days. The child suffers from occasional fever every fifteen to twenty days. There were no malarial parasites in the blood. The liver and spleen were normal. On looking at the area with a high-power hand-lens, the appearance of the affected skin was as if it were distended with fine saccules of serous fluid throughout the whole thickness of the skin. By this serous sacculation the affected area was raised  $\frac{1}{4}$  of an inch above the surrounding normal tissues. No enlarged glands were noted in the neck.

Films were frequently made from the spot, by puncturing the area in the centre, also in the indurated margin. The blood clotted rapidly. The wounds rapidly healed. There were myriads of free parasites and the mononuclear cells were distended as in the previous cases.

One other person in the house was affected with a similar lesion.

CASE V.—Minnie, daughter of W., native Christian, Gujrat, aged 1 year 8 months. Patient presented what looked exactly like herpetic affection of the mucous surface of the upper lip. However, where the upper edge of the spot met the skin of the upper lip the characteristic slightly indurated red margin was seen. The child looked very ill. The patient's mother states the spot first started one year previously as a small itching spot, which gradually grew.

History of constant fever attacks.—Patient has looked ill for months. Spleen and liver normal. Blood taken from the affected area clots rapidly and presents a similar condition to Case IV. These two last cases have lived in the same house since the birth of Case V.

CASE VI.—Abdul Rahman, a Pathan boy from Kohat, aged 10 years. Patient presented a straw-coloured, hairless, raised area on the tip of the nose, which had spread symmetrically on each side towards the nostrils. The edges of the affected area was covered with papery epithelial scales. The fine thickened margin of the affection seen in previous cases was well marked. The history of the case was confirmed by the political naib tahsildar, who brought the patient to the Institute. The child's attendant was well acquainted with the progress of the affection as he was a friend of the patient's parents.

Fourteen months previously the sore commenced as a small itching khaki-coloured spot. It spread for some three weeks fairly rapidly, then remained practically in the same condition as when it first came under examination.

The nose was slightly enlarged, the affected area was anaesthetic. No enlarged glands were found. The spleen and liver were of normal size. There was no history of fever. None of the child's relations were infected.

On puncturing the area and making films, the same conditions as in previous cases were noted. The blood clotted rapidly. There were no parasites of any kind in the peripheral blood. Both the patient and his guardian state that three kinds of Oriental sore are recognised in the Kohat district. The first is called 'Spumai,' a form that never ulcerates, and is extremely common. The second a flat ulcer, known locally by the name 'Aurangzebe phora,' and by Englishmen as 'Frontier sore.' The third is a deep-seated boil, with a deep-pointed core, this is comparatively rare, and is called locally 'Naroo.' The native treatment for the non-ulcerating type is to apply a wafer of flour soaked in hot oil to the affected site and its immediate vicinity as hot as the patient can bear it. The native opinion around Kohat is that the lesion follows on the bite of the sand-fly, and appears most commonly at the commencement of the hot weather.

CASE VII.—Major C., British officer serving on the Aden Boundary Commission. A small typical non-ulcerating Oriental sore developed on the front of the wrist and lasted for eight months, when it was excised and the area painted with pure carbolic acid. The sore first developed when the Commission encamped at Sanawi at the

foot of the Jehaf plateau in South Arabia. Two Sepoys, one other British officer and a native servant developed similar sores at this camping site. The water supply of the camp was obtained from two wells situated in a belt of tamarisk, where the camels were kept during the breeding season. Films made from these cases showed swarms of typical parasites. The blood clotted rapidly, and though repeatedly punctured there never was any tendency on the part of the sores to break down.

CASE VIII.—Gunner K, 4th Battery, Mian Meer. Patient presents multiple infection with what seems to be the flat ulcerating type of Oriental sore. There are eight small flat ulcers on and around the left knee, sixteen similar sores on the left leg, front of left foot and round the left ankle joint. Seven small ulcers occur round right knee on right foot and leg, one larger flat ulcer above the right buttock. There are twenty-five old white scars on left leg and foot, twenty-two scars on right leg and foot. One small ulcer presented itself on the inner side of the left eyebrow. Patient has only been stationed at Mian Meer. There was no history of syphilis. The first ulcer appeared eleven months previously. Examinations of all the ulcers showed bacterial invasion alone. The peripheral blood showed benign tertian parasites but no other protozoa. The blood clotted slightly more rapidly than normal blood, but much less rapidly than in the previous seven cases. None of the patient's comrades are or have lately been similarly affected.

The point of interest in the first set of cases as compared with the last are:—

1. Non-ulcerating character.
2. Increased coagulability of blood.
3. High constant infection of mononuclear cells.
4. Rare infection of polynuclears.
5. Long history, and appearance of other similar lesions at long intervals.
6. Possible infection from another case in close daily contact.
7. Primary itching followed by anaesthesia.
8. Lesion presents constantly a central smooth surface, papery epithelial scales at periphery, margin indurated and visible to the naked eye.
9. Liver and spleen unaffected.
10. Possible exacerbations at irregular periods.
11. General malaise.
12. Occurs at all ages and in Europeans as well as natives.
13. First area affected usually on exposed surface.

The method of staining adopted for all smears and films from the tissues of the patient or culture tubes was as follows:—To 10 c.c. distilled water add 12 drops of ripened Giemsa stain, shake, and allow to act on films for twenty minutes. Then rinse each film with tap water and stain in a watery solution of cosin 1 in 50,000, until the film, which was dark purple, has changed to a rosy violet,

and the erythrocytes are seen under a low power to be of a rosy pink colour.

The films should be fixed in methyl alcohol for five minutes and blotted dry before the stain is applied.

The best method of making films from culture tubes is described in my paper, 'British Medical Journal,' Sept. 11, 1909, page 650. In a differential leucocyte count made from the affected areas as compared with the peripheral and normal blood, the result in all cases showing protozoal infection presented a marked increase in the polymorphonuclears. Difficulty was experienced in making evenly spread films, as the blood drawn from the lesions clotted in a few seconds. No pain is felt whilst boring into the lesion with a sterile glass pipette until the point has passed through the anaesthetic layer into the deeper tissues, about a quarter of an inch from the surface. As a rule mononuclear cells are found distended with oval parasites; polymorphonuclear cells rarely included any parasites. Many free and dividing forms were seen amongst the cells. These results are very similar to those seen by Thomson and Balfour in Egypt.

The following work on the developmental forms of the parasite of non-ulcerating Oriental sore is based on the material taken from a series of cases in India during the last eighteen months. Flagellated forms were first obtained in November, 1908.

In order to obtain development of the parasites of non-ulcerating Oriental sore the method advocated in September, 1909, is the most certain. To four units of clear non-activated human serum add four units of non-activated red blood cells and mix freely in a bulbed pipette with three units of a mixture of sterilised citrate 10 per cent. and salt 0.75 per cent. When the mixture is complete add four units of a similar citrate solution which has been heavily infected with organisms expressed from a puncture of the infected area. By passing a fine glass tube under the skin surface in all directions from one point of puncture, a free discharge of straw-coloured fluid and blood is easily obtained on pressure, and contamination is reduced to its minimum point. The fluid expressed must be collected and discharged into the citrate as soon as possible, as it clots rapidly. It is necessary to aerate the

contents of the culture each day by drawing air up the stem of the pipette and shaking the mixture in the bulb. The cultures should be kept at 22° C. Under such conditions flagellates appear in forty-eight hours, living symbiotically with masses of cocci and bacteria. These flagellated forms increase rapidly up to 120 hours, and then degeneration processes set in.

In seventy-two hours flagellated organisms of two types are frequently found singly and in pairs. One is of monadine form and stains blue, the other is oval or circular, and stains rosy pink. In ninety-six hours large clusters of rosy pink bodies are found, some flagellated, others not. Amongst such clusters a smaller number of blue staining monadine flagellates are usually seen.

The process of development in culture is as follows:—The minute parasites packed within the mononuclear cells liberate themselves, and rapidly multiply, dividing by simple fission. Each daughter cell grows to the size of the parent cell and divides into two or more grand-daughter parasites. After a series of such divisions, differentiation takes place as revealed by staining films from cultures, certain of the parasites now stain a fine rosy tint. These rose-staining parasites possess but little nucleus or chromatin, they slowly enlarge and a flagellum is extruded from the extra-nuclear centrosome. The other type of parasite develops into an oval pyriform body, which increases in size and divides by fission several times. A flagellum with three plications is extruded from the extra-nuclear centrosome, and the flagellated daughter cell breaks away from a tangle of pale rose-staining material to which these parasites are frequently found attached. The flagellum is usually the same length as the body of the monadine parasites. The zooglea mass of rose-staining material above mentioned seems to have been extruded by the parasites as a protective measure to enable them to fix themselves to one small area whilst development and division by fission takes place. It is no uncommon thing to find groups of many hundreds of parasites in all stages of development, thus differing from the parasite of Delhi boil worked at by Row in 1908 and 1909 in non-acidulated human serum. Surrounding such groups masses of bacilli and cocci are seen growing symbiotically. Groups of eight or ten blue monadine flagellates have been seen surrounded by mixed colonies

of germs living in perfect harmony, thus differentiating their specific nature from the allied parasite that gives rise to Kala Azar. Further differences, morphological and cultural, between the three parasites will be dealt with later.

A curious feature in cultures of non-ulcerating Oriental sore, first noted by me in October, 1909, is the constant occurrence of enormous clusters of what seem at first sight to be giant cocci. These bodies stain purple, and have often a reddish margin or film round them. They vary from forms the same size as an erythrocyte to smaller forms, altogether like cocci, diplococci, etc. This suspicious and interesting point has since been confirmed by Thomson and Balfour in their work on non-ulcerating Oriental sore in Egypt. In the description of material from an affected area on the neck, they note in addition to the parasites found free and in mononuclear cells groups of what seem to be large cocci, also a number of pale blue homogeneous structureless masses—a condition which is seen to occur also in cultures of the parasite from the intestinal tract of *Erthesina fullo*, to be described later. In material from affected areas on the thigh similar coccoidal bodies were found. These observers describe these blue coccoidal bodies as four to six times the size of the small cocci present. They stain feebly in their centres, often present unstained areas, occur in clumps or pairs, and may resemble huge gonococci.

In the light of recent experiments I am of the belief that the life-history of the parasite of non-ulcerating Oriental sore is as follows:—

The cockle-shaped form found in the tissues and mononuclear cells represent the form of the parasite which multiplies in the cells of the host by simple fission. In their earliest form they are seen as an exceedingly minute protoplasmal ring, containing a dot-like nucleus. Such forms are occasionally seen amongst the more maturely developed forms.

After a series of divisions by simple fission in cultures, what would seem to be sexual elements are formed, which stain differently. These seem to pair with interchange of elements. From this point the cycle becomes obscure, and light alone is thrown by observations on the life cycle of a similar parasite to be



subsequently described. If dot-like forms are released from the female cell, as recently seen in *Trypanosoma gambiense*, on examination of infected salivary glands in the intermediate host, the tsetse, these elemental forms might well be the minute bodies occasionally seen in infected mononuclears.

With a view to throwing light on these points the subsequent series of experiments were performed.

Before describing these, a few remarks on the insect host are necessary.

The order of the Rhynchota is divided into three main sub-orders: the Heteroptera, Homoptera, and Phytophthires.

The sub-order Heteroptera is divided into two series. The Gymnocerata having conspicuous antennae and the Cryptocera having their antennae more or less concealed.

The Pentatominae is one of the most extensive of the nineteen sub-families of the Gymnocerata, and includes the largest number of common species. *Erthesina fullo* and *Halys dentatus* are two speckled drab-coloured species commonly found in India, and supposed by competent authorities, such as Maxwell Lefroy to be predacious habitually or occasionally. This insect is widely distributed throughout India, Burma, Assam, Java, Japan, Formosa, Hainan, China and Ceylon. On hatching from the egg, there are four nymphal instars, lasting roughly about a month before the adult stage is reached.

Mature specimens of *Erthesina fullo* are common in the Himalayas throughout the year. Larvae and nymphs are most frequently seen in the spring. The mature insect is readily attracted by light at night and enters houses freely. The younger forms can only be taken on the bark of trees, and have not been noticed to frequent human habitations. In connection with Rhynchota of similar habits, it is as well to recall Donovan's observations in Madras, where he noted *Conorhinus rubrofasciatus* a member of the Acanthaspidinae is a common blood-sucking insect of local distribution found at night, whose nymphs frequent corners and crevices in houses. This insect, though not commensurate in its range with the occurrence of Kala Azar, is of extreme interest, as both nymphs and adults suck human blood if opportunity is afforded. Other Rhynchota with similar habits are

*C. infestans*, fed by Darwin on his blood, and *C. sanguisiga*, found in Arizona, in which latter case the site of puncture from which the insect has sucked human blood becomes painful, inflammation and even pus formation ensuing.

The point of this seeming discursion will be seen later. Forty-three adult specimens of *Erthesina fullo* were dissected, and the contents of their intestinal tract examined. In forty-one insects the crop contained large numbers of a crithidial organism freely motile. In one the infection was scanty, and one insect was found negative.

A series of nymphs and adult specimens of *Erthesina fullo* were dissected, and it was found that infection of the intestinal tract was common in all stages of the insect's life history. No eggs were examined. A few specimens of *Halys dentatus* and another species, as yet undetermined, were dissected and found negative, an interesting point showing the selective preference shown by the parasite for one species of insect. The further selective preference shown by the parasite of *Erthesina fullo* for human blood in culture as compared with blood of other animals is highly suggestive, and opens a large field for research work to those interested in discovering the definitive hosts that transmit the parasites of Kala Azar and the forms of Oriental sore to man.

Adult specimens of *Erthesina fullo* fed readily on my blood, citrated, when it was presented to them. A series of insects were fed thus twice a week through the winter and thrived on the diet.

Stained films prepared from the gut contents, showed all stages of the parasite, from the small cockle-shaped form simulating *Leishmania* to pyriform and flagellated bodies of two varieties as seen in non-ulcerating Oriental sore. The parasites were found living symbiotically with myriads of cocci and bacteria in the crop and intestine.

First series of culture experiments:—The crop of an infected insect, having been dissected out, was placed in a drop of citrate solution, 1 per cent. with 0.75 per cent. salt, and kept in a moist chamber for twenty-four hours. When the contents were examined the same conditions were noted as occurred in material from freshly dissected insects. There was, however, a marked increase in the number of the flagellate forms.

Ten drops of citrate solution were infected with the contents of the crop of two infected insects, and a series of cultures were made with bulbed pipettes as before.

A. A mixture of human blood citrate solution and infected citrate in equal units.

B. Ditto.

C. Human serum and infected citrate, equal units.

A similar series of culture tubes were made up, in which the blood from the rat, guinea-pig, rabbit, fowl and lizard (*Agama tuberculata*) was put up with equal units of citrate solution and infected citrate.

A similar series of serum cultures from these latter were also put up, and controls in each case made without infected citrate. The result of these experiments is rather striking, showing the selective preference of this parasite for human blood. In a culture of this parasite in citrated human blood the whole culture was found seething with active flagellates in twenty-four hours. This rapid increase was still more marked in forty-eight hours. Large groups of flagellates and parasites in all stages were common. After 192 hours a few live flagellating parasites were seen, most of them were motionless. In fresh preparations put up under a vaseline ringed cover-slip, the same process of multiplication was watched daily. The flagellated parasites were found to arrange themselves in large clusters around air bubbles with their flagella attached to the under surface of the bubble. Death, apparently due to want of air, was seen to occur in this confined area, usually in 168 hours.

The formation of pairs of monadine flagellate and circular or oval bodies was noted frequently, a condition checked by other workers in the laboratory.

In human serum the parasites vanished in forty-eight hours, a few cyst-like bodies with thick capsules alone were seen.

In blood and serum cultures from the common grey rat, no increase in the number of the parasites took place. They became motionless in four hours and had vanished in ninety-six hours. In blood and serum cultures from the white rat the parasites were found still motile after twenty-four hours, but were all motionless,

and the slide showed no increase in the parasites in forty-eight hours. In cultures made from the blood and serum of the guinea-pig, fowl and lizard there was no increase, a few parasites only were found dead and motionless after twenty-four hours. In lizard and fowl's blood and serum the parasites became motionless in four hours.

In the blood of the rabbit many single flagellates were seen actively motile up to ninety-six hours. There were no groups formed by multiplication of the parasites. After 120 hours the parasites became sluggish and finally motionless. Stained films showed no evidence of multiplication at any time.

Several interesting points were noted both in the fresh and in stained preparations from the culture tubes.

In cultures of the parasite in human blood it was frequently seen that at about the end of twenty-four hours pairs of parasites of different form were found constantly in apposition. The one a monadine flagellated body, the other a small parasite either boat-shaped, oval, or round, which was apposed to the monadine form in the neighbourhood of the nucleus in the case of the oval form, and often near the commencement of the flagellum in the case of the early boat shaped type. The character of the two forms, as shown in the plates, will be seen to be of the same nature as seen in the parasites of non-ulcerating Oriental sore during the stage of numerical increase in culture tubes. Stained preparations showed the monadine form stained bluish, the cyst-like body in connection with it rosy pink and with a flagellum often attached. In the case of the minute boat-shaped form this usually stained a bright blue in the early stage, and contains the same structural elements as the early form of *Leishmania*. Increase in the number of the parasites continued up to 144 hours in citrated cultures of human blood. After 196 hours but few motile flagellates were found, the majority of the parasites were motionless, and many showed commencing disintegration.

The monadine flagellate type had a characteristic movement. The anterior part of the parasite moved to and fro with the flagellum. The posterior one-third rarely is distorted. The flagellum would give five or six flicks then the whole parasite would vibrate, next the body of the parasite rapidly curves in **S** forms

for a few seconds, and becomes quiescent for a short period. The tip of the flagellum is applied by the parasite to the edge of the red cell frequently as if deriving nutriment from its contents.

The flagellated oval or round form of the parasite streams slowly along the field of the microscope propelled by the flagellum.

A detailed description of the parasite when stained takes up space, and is best elucidated by accurate drawings, the morphological details of the various stages of this parasite are shown in the accompanying plates, all drawn with the large Abbé camera lucida and Bernhard's drawing table.

A point of interest, and which may throw light on the rosy zooglea formation seen in culture groups of the parasites of Kala Azar and the varieties of Oriental sore, is the formation of what I propose to term 'anchor cords,' first noted in the monadine flagellate form of this parasite.

On putting up this parasite with citrated guinea-pig's blood, as above, and examining fresh-ringed cover-slip preparations after four hours a curious condition was seen for the first time. A group of four monadine flagellates were noticed to leave a certain refractile glass-like spot on the slide, and with the aid of their flagella explore the various channels that lay between adjacent groups of red corpuscles. It was noticed they invariably returned to the same spot after their excursions, often almost beyond the limit of the field of the microscope. When the light from the condenser was markedly reduced it was now noted that the posterior end of each parasite was connected by a fine thread to a small spot in a minute patch of sticky material adhering to the slide, which material was faintly ground-glass in appearance. These 'anchor cords' were extremely fine and highly elastic, and enabled the free flagellated parasite to wander far from the central spot, but yet return to its starting point from any angle with absolute certainty. There was no coiling up of these threads as the parasites returned to their common central point in the zooglea mass, they contracted and expanded with the requirements of the parasite, but seemed to have an equal length as regards the extreme limit of distance to which the parasites are capable of wandering. Such an apparatus can have only one or both objects in view: exploration for food material or the opposite sex.

It is probable this morphological detail occurs at this stage in the life-history of other allied flagellates and has hitherto escaped notice. These 'anchor cords' are extremely difficult to focus, but once located were shown to and detected by two other workers in the laboratory.

A series of adult *Erthesina fullo* were placed in a sterilised glass box and fed only on distilled water in a pledget of sterile wool for three winter months. The air in the box was changed once a week. This shows the vitality of the insect and its parasite. On dissection, the crop of each insect was found distended to about the size of a pea, with clear greenish fluid.

Examination of this fluid revealed numerous clumps of pyriform forms and free flagellates. Earlier forms were seen in large numbers.

A similar series of experiments with a series of bloods as before gave the same results.

On separating a series of insects fed only on water for three months, an insect was noted to defaecate a large quantity of dark, watery, greenish fluid. On examination of this fluid it was found to contain large numbers of parasites in all stages, the flagellate forms were, however, all motionless.

On making citrated blood cultures of the fluid with human, rabbit, fowl, guinea-pig, and white rat's blood, and preparing vaseline-ringed cover-slip preparations, it was noted the flagellates previously motionless and seemingly dead had all become freely active in four hours.

In the case of blood cultures from the rabbit, fowl, and guinea-pig with this material, the flagellates again were found motionless in twenty-four hours, and disintegrating. In the blood of the white rat flagellating parasites were seen after twenty-four hours, but dead in forty-eight hours.

In human blood, however, it is interesting to note there was an enormous increase in the numbers of the parasites by the end of forty-eight hours. Many pairs of monadine flagellates and cyst-like bodies were seen. Parasites in all stages of development were found increasing in numbers up to 144 hours; from this point they grew more sluggish in their movements and became motionless and degenerated.

From a primary culture of the parasite in human blood which showed flagellates after ninety-six hours, a sub-culture was made into fresh citrated human blood.

This sub-culture showed freely moving flagellates in 120 hours, none could be found after 168 hours.

That mature insects void the parasite in all its stages indicates the method of infection of others of the same species. It is not uncommon to find several of these insects huddled together in one spot. The fact that motionless and seemingly dead flagellar forms capable of revivifying in a suitable medium and multiplying in it are voided, shows that infection of other insects need not necessarily be confined to the infection by cyst forms, a commonly accepted belief amongst some workers at this branch of protozoology.

In addition to the stages in the life-history of the parasite mentioned above, we note that in the ileum, and below the crop, many cyst-like bodies occur with granular contents. These, if a phase in the life cycle of the parasite, and not another gut organism, would seem to be the stage previous to the liberation of the earliest form of the parasite.

To review the points of similarity between the parasite causing non-ulcerating Oriental sore and that infesting the intestinal tract of *Erthesina fullo*.

They both multiply and develop pyriform forms, monadine and circular or oval flagellated forms, in a culture of human blood acidulated with sodium citrate. Neither parasite will develop in an alkaline medium. They both live and develop symbiotically with masses of cocci and bacteria.

Bodies like giant cocci and bluish homogeneous bodies are found in cultures of both parasites.

Pairs of dissimilarly shaped and staining flagellated parasites are seen in both cases, whilst in the parasite of *Erthesina fullo* cyst-like bodies have been found suggestive of the formation of multiple early forms from a fertilised female.

The crithidial parasite of *Erthesina fullo*, when examined in the living condition, affords many interesting points likely to throw light on the life cycle of allied parasites. When a drop of human blood-culture fluid has been kept under a vaseline-ringed cover-slip for eighteen hours, and a few small bubbles of air

similarly enclosed amongst the medium, the fully formed monadine flagellates are found often to have arranged themselves in clusters around the edge of the air space, with the tips of their flagella pointing towards its centre. The parasites do not leave the bubble, but remain vigorously swaying to and fro, as if having found air they fear to leave it. In early fresh-infected cultures of human blood one of the most striking and frequent features is the linking up of a small motile boat-shaped body to the fully-formed crithidial monadine flagellate. The smaller body is found frequently apposed to the body of the former at the root of the flagellum. It may, however, be apposed opposite the nucleus or even on the flagellum itself. Once the small body has fixed itself to the larger parasite it seems to become securely stuck to the site, and accompanies the flagellate monadine in its peregrinations without being dislodged. Such pairs are commonest seen in the first period of rapid multiplication of the parasites.

The monadine flagellate presents, as a rule, pointed ends, the anterior flagellated end tapers finely, and in many cases passes a marked way up the flagellum. Occasionally the monadine form presents a globose posterior end, looking like a pouch filled with numerous brightly refractile granules.

The monadine form presents in the fresh condition an easily seen granular nucleus, a clear centrosome with indications of a surrounding vacuole. Small golden granules are commonly seen at either end of the body of the parasite, but usually occur in greatest number in the posterior portion.

When the monadine forms die, one often sees in fresh specimens a clear vacuole-like body in the centre of the parasite. Occasionally one sees evidence of a kind of prolongation of the flagellum into the body of the parasite, giving it a bladed screw-like appearance. Other parasites after death are globose in their centres, this globular area is clear as a rule, whilst collections of golden granules are seen at the posterior end and round a dark pigmented area, anterior to the nucleus, probably the site of the extra-nuclear centrosome.

In the case of the monadine form found in apposition with cyst-like bodies at the level of, or in the neighbourhood of the nucleus of the former, a detailed description can be given, as it is



based on a large series of observations by myself and other workers in the laboratory.

The smaller rounded cyst-like body may, or may not, present a flagellum arising from a clear staining spot, the extra-nuclear centrosome. The body of the parasite in favourable cases is seen to have (1) a fine, clearly defined, smooth capsule; (2) a comparatively large area of scattered granular material; (3) the nucleus a large dark spot is seen in one portion of the cell. Round this nucleus are often arranged a ring of fine dark dots.

A reniform ground-glass area, looking like a vacuole, is occasionally seen separated from the cuticle of the cell by a series of dark dots, seemingly equidistant from each other. These dots vary in number and in position, they may be found in a loose clump at one side of the cell.

The monadine form may or may not present a certain degree of globular enlargement at the point of contact between the two bodies. Where the small cell lies apposed at the level of the nucleus this globular enlargement has been most frequently seen. The nucleus of the monadine form has a ground-glass appearance, and its centre is usually situated at the junction of the anterior and middle thirds of the body of the parasite. The flagellum is usually about the same length as the body of the parasite, and presents two to four wide undulations. The root of the flagellum passes down the sinuous tapering anterior point of the parasite to end in the immediate vicinity of the extra-nuclear centrosome. When this latter is seen clearly, it is usually recognised as a brilliant clear oval spot, with a dark edge to it, the whole surrounded by a fine band of ground-glass-like material.

Between the extra-nuclear centrosome and the filamentous anterior extremity an area thickly sown with fine granules is seen. The posterior third of the parasite may show two definite dark refractive dots or a varying number of granules. Occasionally a cyst-like body, non-flagellated, showing a reniform vacuole and a large number of granules, as it were boiling within it, has been seen. Further experimental work on the later stages of the mature parasites is necessary, and these cyst bodies are recorded without comment.

## EXPLANATION OF PLATES

## PLATE III

I.—Shows pairs of flagellated organisms from a culture tube of non-ulcerating Oriental sore. These stain differently; the blue monadine form\*, the rosy pink oval form.

These organisms are in relation with a group of large coccoidal bodies, whose nuclei stain dark-bluish purple, and are often surrounded by a fine zone of cytoplasm, staining pink.

Film made after seventy-two hours' culture at 22° C.

II.—A cluster of parasites in all stages of development in a ninety-six hours' culture of non-ulcerating Oriental sore kept at 20° C.

The parasites are seen living symbiotically with bacteria and cocci. A large clump of parasites showing early differentiation into the two forms which stain differently and present flagella seen at the edge of the clump.

Two rosy-staining zooglea masses are shown.

III.—Pairs of bodies seen in a culture of non-ulcerating Oriental sore seventy-two hours old.

IV.—A group of rosy bodies, amongst which a few blue-staining monadine forms are seen. Note the group of coccoidal bodies, also the curious apposition of a flagellated rosy body to that of a blue monadine flagellate at the level of the nucleus.

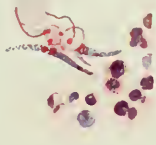
From a culture of non-ulcerating Oriental sore ninety-six hours old.

V.—Blue monadine flagellates, dividing forms, also others in conjunction with rosy bodies.

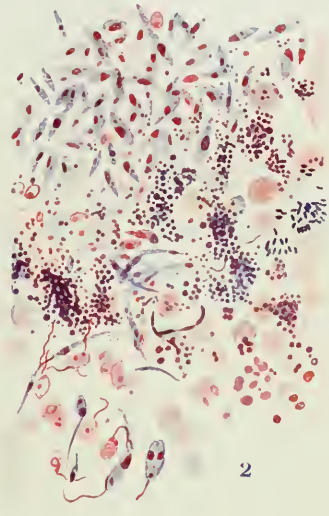
Note a globose enlargement in a blue monadine form opposite the point of apposition of a flagellated rosy body.

Drawn from a culture of non-ulcerating Oriental sore ninety-six hours old.

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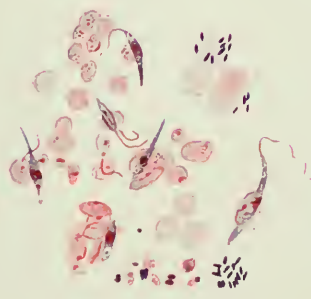
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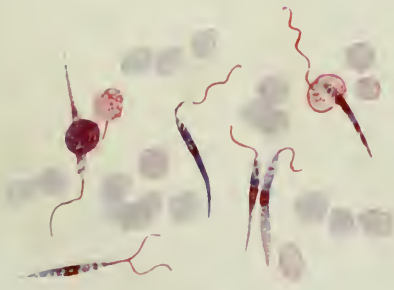
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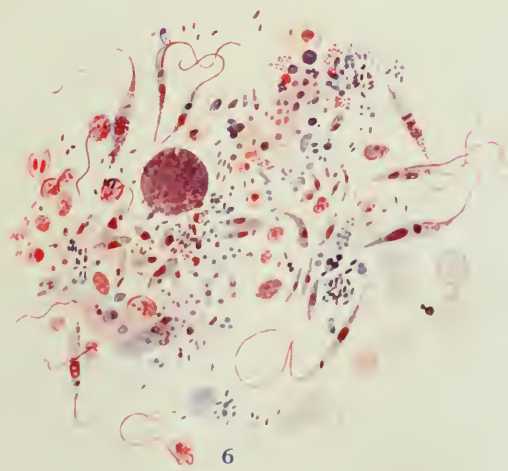
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6

VI.—A group of organisms drawn from a culture of parasites from *Erthesina fullo* after seventy-two hours.

Note the parasites are seen in all stages of development, large blue coccoidal masses are seen attached to two rosy zooglea masses. The morphological details of the bluish monadine form are chiefly crithidial in type. The rosy bodies simulate those found in non-ulcerating Oriental sore. The parasites are seen living symbiotically with bacteria.

## PLATE IV.

Series of drawings of living specimens of the gut parasite from *Erthesina fullo* in blood cultures. I-XII and No. XIX drawn with  $1/12$  objective No. 2 eye-piece, XIII-XVIII and No. XX drawn with  $1/12$  objective and No. 4 eye-piece.

I.—Oval body showing nucleus, nucleolus and vacuole.

II and III.—Monadine parasites (*b*) with small oval or boat-shaped bodies, (*a*) apposed at the root of the flagellum.

IV and V.—Flagellated large oval parasites.

VI.—Monadine flagellate (*b*), and small oval body (*a*). Note granular posterior and anterior ends of the former.

VII and VIII.—Flagellate parasite with the posterior ends globose and filled with granules.

IX and X.—A monadine flagellate dividing by fission from the anterior end of the parasite.

XI.—Monadine flagellate with globose granular centre.

XII.—Edge of an air bubble in a cover-glass preparation showing the arrangement of an aggregate of monadine flagellates, with their flagellae towards the centre of the lower surface of the bubble.

XIII.—A pair of parasites, the one (*b*) a monadine flagellate, the other (*a*) a cyst-like body unflagellated apposed at the level of the nucleus of the monadine parasite. The monadine parasite (*b*) presents a ground-glass-like nucleus about its centre. The posterior end of the parasite presents two dark dots. The anterior portion of the parasite presents fine granules and a small, clear, dark-edged oval area surrounded by a fine vacuole. Occasionally the root of the flagellum can be traced ending in the neighbourhood of the vacuolar area.

The oval parasite (*a*) presents a fine capsule. To one side lies a large dark dot surrounded by a ring of fine dots. These adjoin a reniform ground-glass-like area, seemingly like a faint vacuole. This latter is separated from the capsule of the parasite by a line of equidistant dots of equal size.



XIV, XV, XVI and XVII.—Similar pairs of parasites, the oval parasite flagellated or not.

XVIII.—Cyst-like bodies packed with small golden granules, which seemed to boil within the capsule.

XIX.—Four monadine flagellates with acicular posterior extremities, each parasite attached by a fine elastic cord to a spot in a mass of zooglea material.

XX.—A parasite showing the posterior end of the parasite contains clear fine granules.

A and B show two positions of the parasite leaving the zooglea clear mass with the flagellated anterior extremity forwards.

*b.*—The fine elastic posterior 'anchor cord.'